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Original Article

Ginsenoside Rg2 attenuated myocardial injury induced by isoproterenol in rats

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Abstract: The present study was designed to investigate the effect of ginsenoside Rg2 on myocardial injury induced by isoproterenol in rats. Sprague-Dawley rats were subcutaneously injected with isoproterenol (20 mg/kg). Creatine kinase-MB (CK-MB) activity and troponin T level in serum, antioxidative parameters, and inflammatory cytokines in left ventricles were measured. Histopathological examination of the left ventricles was performed. CK-MB activity and troponin T level in isoproterenol-treated rats were significantly increased. Isoproterenol induced increases of the contents of malondialdehyde (MDA), tumor necrosis-α (TNF-α), interleukin-1β (IL-1β), and decreases of the activities of superoxide dismutase (SOD), catalase, glutathione reductase (GR) and level of glutathione (GSH) in the left ventricles. Ginsenoside Rg2 reduced not only CK-MB activity and troponin T level but also myonecrosis, edema, and infiltration of inflammatory cells. Ginsenoside Rg2 inhibited the increases of MDA, TNF-α, IL-1β contents and the decreases of SOD, catalase, GR, and GSH in the left ventricles. The present findings demonstrated that ginsenoside Rg2 might have a potential benefit in preventing ischemic heart diseases.

Keywords: Ginsenoside Rg2, myocardial injury, antioxidant, anti-inflammatory

Introduction

Myocardial infarction is an acute condition of myocardium necrosis that is caused by an imbalance between the coronary blood supply and the demand of the myocardium [1]. Although great improvements have been made in medical care and clinical nursing, myocardial infarction still remains the leading cause of disability and mortality worldwide, even lasting to the year of 2020 [2]. Recent research provides evidence that oxidative stress, induced by increased generation of reactive oxygen species, plays a role in the pathogenesis of myocardial infarction [3]. The increased oxygen stress damages the membrane lipids, carbohydrates, DNA and proteins, thereby leading to cellular destruction, energy deficiency, and the acceleration of cell death [4]. Inflammation has been recognized as a critical factor in cardiovascular disease, and numerous anti-inflammatory drugs have been demonstrated as effective in attenuating myocardial injury [5]. Thus, therapeutic intervention with antioxidant and anti-inflammatory treatment may prevent the deleterious changes and attenuate myocardial dysfunctions induced by myocardial infarction.

Catecholamines overproduction is a great risk for cardiac dysfunction. It has been reported that supraphysiological levels of catecholamine in plasma may produce cardiac muscle dysfunction by affecting myocardial energy metabolism [6]. Isoproterenol (ISO) is a synthetic catecholamine that causes rigorous stress to the myocardium. The pathophysiological and morphological alterations of myocardium following ISO administration in rats have been proven to be comparable with those taking place in humans. Thus ISO-treated experimental rats are a well-recognized standard model used to study the cardiac functions and beneficial effects of many drugs [7].

Ginseng, the root of *Panax ginseng* C.A. Meyer (Araliaceae), is a traditional medicine in Asian countries. The main active components of *Panax ginseng* are ginsenosides. Ginsenosides have been shown to have a variety of beneficial consequences, including anti-inflammatory, antioxidant, anti-cancer effects, anti-mutagen-

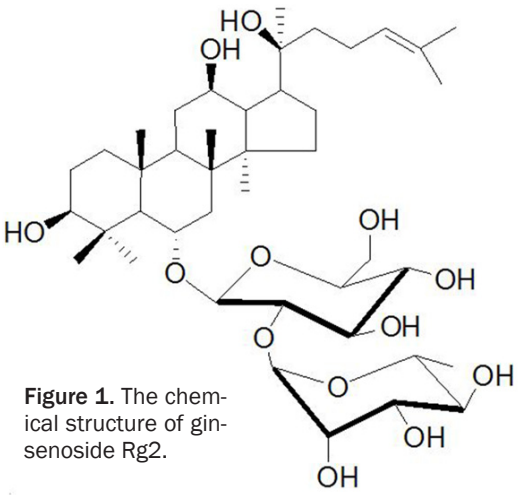

Figure 1. The chemical structure of ginsenoside Rg2.

ic, anti-aging activities and immunomodulatory action [8]. Ginsenoside Rg2 is a protopanaxatriol-type compound that is one of the major active components in the root and stem leaves of ginseng. Ginsenoside Rg2 has a neuroprotective effect against hypoxia-induced neuronal damage in hippocampal neurons mediated by eliminating the free radicals and increasing the activities of anti-oxidative enzymes [9]. Ginsenoside Rg2 also attenuated glutamate-induced neurotoxic effects through the mechanism related to anti-oxidation [10]. Ginsenoside Rg2 has a strong ability in ameliorating oxidative damages induced by H_2O_2 [11]. In the present study, we evaluate the effect of ginsenoside Rg2 on myocardial injury induced by isoproterenol in rats.

Materials and methods

Chemicals and reagents

Ginsenoside Rg2 (**Figure 1**) was purchased from Cayman Chemical Company (Michigan, USA). Isoproterenol, thiobarbituric acid, nitroblue tetrazolium, GSSH, NADPH, H_2O_2, and other chemicals (analytical grade) were from Sigma Co. (St. Louis, MO, USA). Creatine kinase-MB (CK-MB) diagnostic biochemical assay kit was obtained from Biosino Biotechnology Company Ltd. (Beijing, China). The ELISA kit of troponin T was purchased from Westang Biomedical Technology Company (Shanghai, China). The ELISA kits of tumor necrosis-α (TNF-α) and interleukin-1β (IL-1β) were obtained from R&D Systems, Inc. (Minneapolis, MN, USA). Carboxymethyl cellulose sodium salt (CMC-Na) was purchased from Shanghai Jinshan Chemical Co., Ltd. (Shanghai, China).

Animals

The experiments were performed according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (publication 86-23, revised in 1986) and were approved by the local Ethics Committee. Male Sprague-Dawley rats weighing 280-300 g were obtained from the Beijing HFK Bioscience Co., Ltd (Beijing, China). The rats were housed in diurnal lighting conditions (12 h/12 h) and allowed free access to food and water for 7 days before the experiment.

Experimental protocols

Animals were randomly divided into four groups of 10 rats each: control group (Control), isoproterenol group (ISO), ginsenoside Rg2 at dose of 2.5 mg/kg group (Rg2 2.5 mg/kg), and ginsenoside Rg2 at dose of 10 mg/kg group (Rg2 10 mg/kg). The rats in the ginsenoside groups were pretreated orally with ginsenoside Rg2 at doses of 2.5 or 10 mg/kg daily for a period of 7 days. The rats in the control and Iso groups were given the same volume of 1% CMC-Na. According to the previous report [12], rats in the ISO and ginsenoside groups were subcutaneously injected with isoproterenol at a dose of 20 mg/kg and at an interval of 24 h for 2 days to induce myocardial injury (on the 6th and 7th day). Animals in the control group were subcutaneously treated with normal saline.

Assay of CK-MB and troponin T

At the 24 h of the last isoproterenol injection, blood samples were collected in test tubes. The samples were then centrifuged (3000 *g*) at 4°C for 10 min. The serum was stored at -80°C for biochemical assay. The activity of CK-MB was evaluated using commercial kits by employing an automatic biochemical analyzer (Leidu Company, Shenzhen, China). The level of troponin T was assayed using an ELISA kit. All samples were measured in duplicate.

Histopathological examination

The left ventricles of 2 rats in each group were fixed in 10% formalin. The specimens embedded with paraffin were cut into 5 μm thick sections and stained with hematoxylin-eosin. An experienced observer who was blind to the treatment under light microscope examined the sections and then photomicrographs were taken.

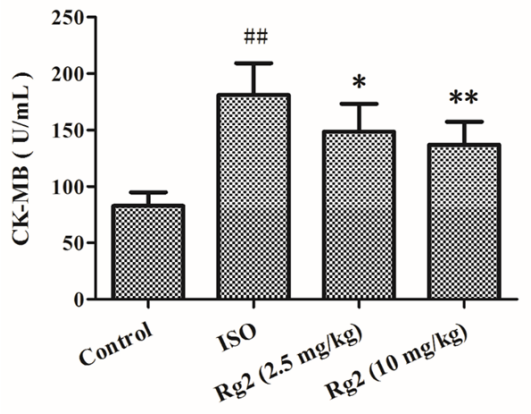

Figure 2. Effect of ginsenoside Rg2 on CK-MB activity in isoproterenol-induced myocardial injury in rats. Data was expressed as the mean ± S.D. (n = 10). The statistical significances of the data were determined using one-way analysis of variance (ANOVA) followed by the least significant difference testing. $^{\#\#}P < 0.01$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with ISO (isoproterenol) group.

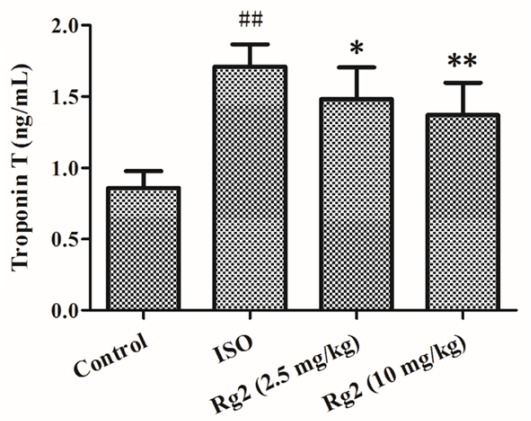

Figure 3. Effect of ginsenoside Rg2 on troponin T level in isoproterenol-induced myocardial injury in rats. Data was expressed as the mean ± S.D. (n = 10). The statistical significances of the data were determined using one-way analysis of variance (ANOVA) followed by the least significant difference testing. $^{\#\#}P < 0.01$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with ISO (isoproterenol) group.

Measurement of malondialdehyde (MDA), superoxide dismutase (SOD), catalase, and glutathione (GSH), glutathione reductase (GR)

The left ventricles of 8 rats in each group were excised and homogenized in 9 volumes of ice-cold saline. The homogenate was centrifuged (4000 *g*) at 4°C for 15 min. The supernatant was collected and stored at -80°C for analysis. The amount of protein in the supernatant was measured according to the previous method [13]. MDA content in the left ventricle was performed according to the previous method [14]. SOD activity in the left ventricle was evaluated by the nitroblue tetrazolium reduction method [15]. Catalase activity in the left ventricle was assayed at 37°C by following the rate of disappearance of H_2O_2 at 240 nm [16]. GSH was measured in the cytosolic fraction fluorimetrically following the previous method [17]. GR activity was calculated by the method of Calberg and Mannervik [18].

Assay of TNF-α and IL-1β

The homogenate of the left ventricle was prepared as described above. TNF-α and IL-1β levels in the supernatant were measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions. Absorbance values were assayed at 450 nm using an ELISA plate reader (Bio-Tek, USA). All samples were measured in duplicate.

Statistical analysis

The data are expressed as the mean ± S.D. The statistical significances of the data were determined using one-way analysis of variance (ANOVA). Then the Least Significant Difference (LSD) testing was applied for comparisons of the difference between 2 groups. The *P* value < 0.05 was considered as statistically significant. Statistical analysis was carried out with SPSS 19.0 software.

Results

Effects of ginsenoside Rg2 on CK-MB activity in isoproterenol-induced myocardial injury rats

Animals challenged with isoproterenol showed a significantly increased activity of CK-MB compared to the control rats ($P < 0.01$). Treatment with ginsenoside Rg2 decreased the activity of CK-MB compared with the rats in ISO group ($P < 0.05$ or $P < 0.01$, **Figure 2**).

Effects of ginsenoside Rg2 on troponin T levels in isoproterenol-induced myocardial injury rats

The troponin T level was significantly increased in isoproterenol-induced myocardial injury of rats as compared to the control rats ($P < 0.01$).

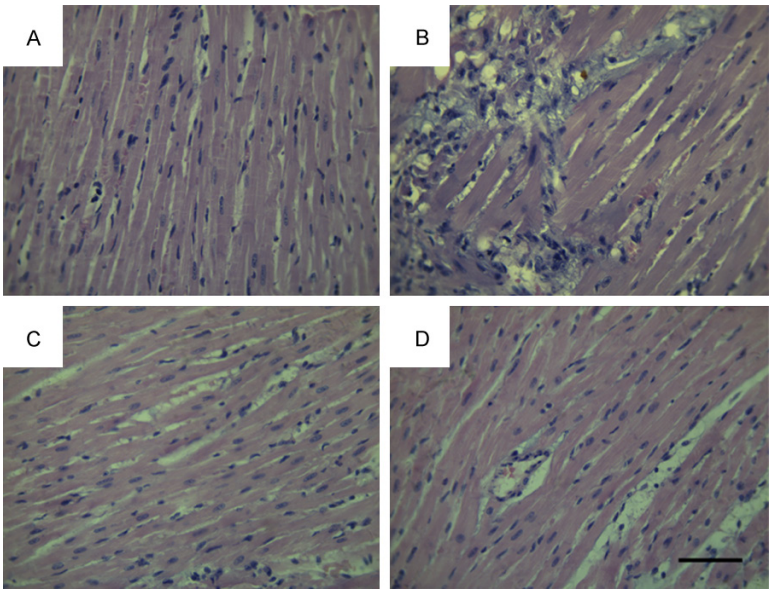

Figure 4. Histopathological examination in isoproterenol-induced cardiotoxicity in rats. A: Control group, B: Isoproterenol group, C: Ginsenoside Rg2 2.5 mg/kg group, D: Ginsenoside Rg2 10 mg/kg group. Control group showing clear integrity of myocardial cell membrane, and no inflammatory cell infiltration. Isoproterenol induced widespread myocardial structure disorder and subendocardial necrosis with edema and inflammatory cells. The rats treated with ginsenoside Rg2 (2.5 or 10 mg/kg) showing mild edema, less inflammatory cells and necrosis. Scale bar = 40 μm.

Effects of ginsenoside Rg2 on the histological changes in the left ventricles

The left ventricles from the control group rats showed a normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils (**Figure 4A**). However, the left ventricles from the isoproterenol-challenged rats displayed widespread myocardial structure disorder and subendocardial necrosis with edema and inflammatory cells (**Figure 4B**). The rats in the ginsenoside Rg2 (2.5 and 10 mg/kg) groups exhibited mild edema, less inflammatory cells and necrosis (**Figure 4C**, **4D**).

Effects of ginsenoside Rg2 on MDA content in isoproterenol-induced myocardial injury rats

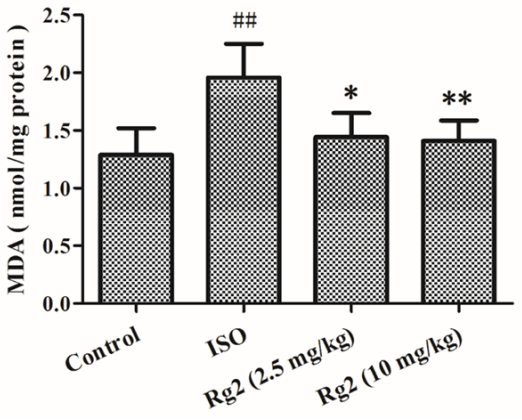

Figure 5. Effect of ginsenoside Rg2 on MDA level in isoproterenol-induced myocardial injury in rats. Data was expressed as the mean ± S.D. (n = 8). The statistical significances of the data were determined using one-way analysis of variance (ANOVA) followed by the least significant difference testing. $^{\#\#}P < 0.01$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with ISO (isoproterenol) group.

Treatment with ginsenoside Rg2 (2.5 and 10 mg/kg) daily for 7 days decreased the change of troponin T levels induced by isoproterenol administration ($P < 0.05$ or $P < 0.01$, **Figure 3**).

Compared with the control group, isoproterenol caused an increase of MDA content in the left ventricles of rats ($P < 0.01$). Administration of ginsenoside Rg2 at a dose of 2.5 and 10 mg/kg attenuated the isoproterenol-induced increase in MDA content ($P < 0.05$ or $P < 0.01$, **Figure 5**).

Effects of ginsenoside Rg2 on the activities of SOD and catalase in isoproterenol-induced myocardial injury rats

The activities of SOD and catalase significantly decreased in isoproterenol-challenged rats as compared to that of the control group ($P < 0.01$). Compared with the isoproterenol group, the activities of SOD and catalase in ginsenoside Rg2 (2.5 and 10 mg/kg) groups significantly increased ($P < 0.05$ or $P < 0.01$, **Figure 6**).

Effects of ginsenoside Rg2 on the GSH levels and GR activity in isoproterenol-induced myocardial injury rats

GSH level and GR activity showed decreases in isoproterenol-challenged animals ($P < 0.01$).

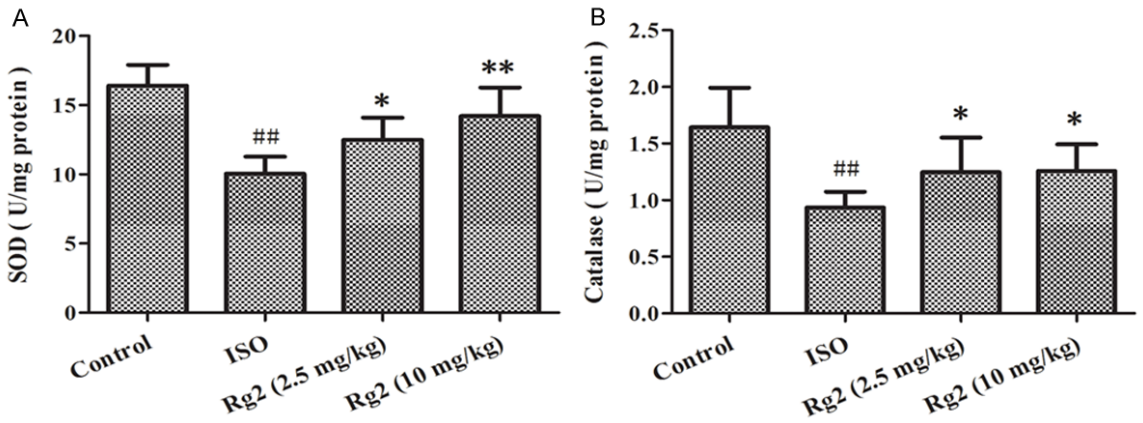

Figure 6. Effect of ginsenoside Rg2 on SOD (A) and catalase (B) activities in isoproterenol-induced myocardial injury in rats. Data was expressed as the mean ± S.D. (n = 8). The statistical significances of the data were determined using one-way analysis of variance (ANOVA) followed by the least significant difference testing. $^{\#\#}P < 0.01$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with ISO (isoproterenol) group.

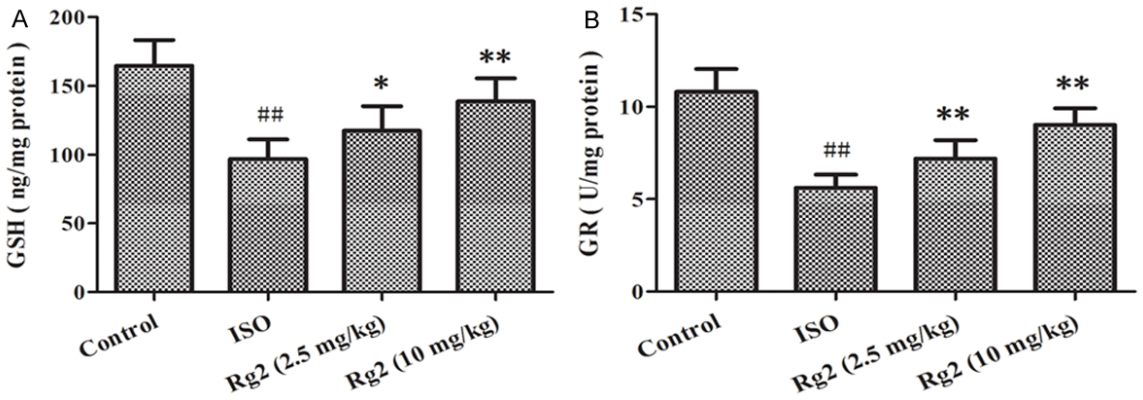

Figure 7. Effect of ginsenoside Rg2 on GSH level (A) and GR activity (B) in isoproterenol-induced myocardial injury in rats. Data was expressed as the mean ± S.D. (n = 8). The statistical significances of the data were determined using one-way analysis of variance (ANOVA) followed by the least significant difference testing. $^{\#\#}P < 0.01$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with ISO (isoproterenol) group.

Compared with the control group, treatment with ginsenoside Rg2 (2.5 and 10 mg/kg) in isoproterenol-challenged rats increased the GSH level and GR activity in the left ventricles ($P < 0.05$ or $P < 0.01$, **Figure 7**).

Effects of ginsenoside Rg2 on TNF-α and IL-1β levels in isoproterenol-induced myocardial injury rats

TNF-αand IL-1β levels significantly increased in the left ventricles of isoproterenol-challenged rats as compared to that of the control group. Compared with the isoproterenol group, the TNF-α and IL-1β levels in the ginsenoside Rg2 (2.5 and 10 mg/kg) groups significantly decreased ($P < 0.05$ or $P < 0.01$, **Figure 8**).

Discussion

This study evaluated the effects of ginsenoside Rg2 treatment on the myocardial injury induced by isoproterenol. We attempted to elucidate the mechanisms of therapeutic efficiency of ginsenoside Rg2 by studying the biochemical markers, histopathological alterations, anti-inflammatory cytokines and the antioxidant defense system. The results suggested that ginsenoside Rg2 had cardioprotective effects in isoproterenol-induced myocardial injury rats.

Isoproterenol-challenged rats had been reported to show many metabolic and morphologic aberrations in the heart tissue that was similar to those observed in humans [19]. Therefore,

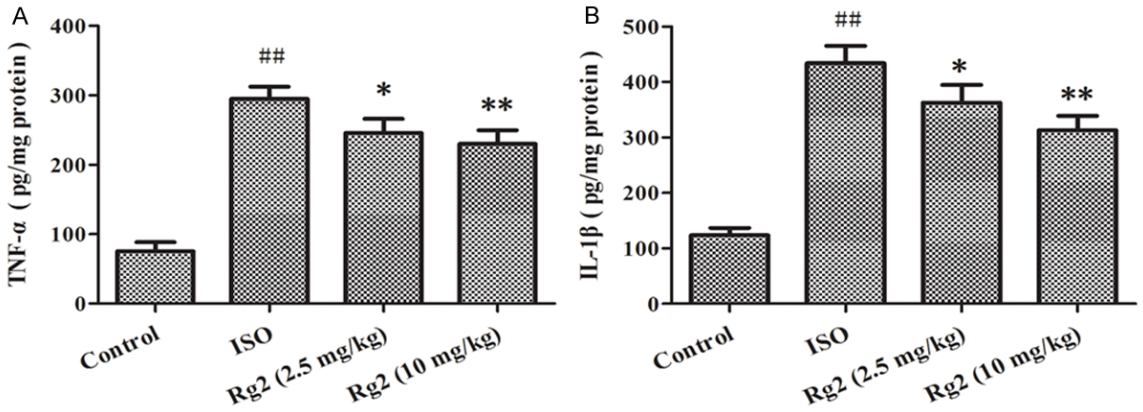

Figure 8. Effect of ginsenoside Rg2 on TNF-α (A) and IL-1β (B) levels in isoproterenol-induced myocardial injury in rats. Data was expressed as the mean ± S.D. (n = 8). The statistical significances of the data were determined using one-way analysis of variance (ANOVA) followed by the least significant difference testing. $^{\#\#}P < 0.01$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with ISO (isoproterenol) group.

isoproterenol has been widely used to evaluate cardioprotective drugs and to study myocardial consequences of ischemic disorders.

Myocardium contains plentiful concentrations of CK-MB. Isoproterenol damaged myocardial tissue and then CK-MB will leak out. Therefore, the CK-MB activity in the serum can reflect the degree of myocardial injury. Cardiac troponins are regulatory proteins that control the calcium-mediated interaction of actin and myosin. An elevated troponin level predicted the risk of cardiac injury and death. A recent clinical study proposed that increasing serum troponin T concentration can be used to identify asymptomatic myocardial damage and predict the risk of myocardial ischemia in patients [20]. The present study showed that the CK-MB activity and troponin T levels were significantly increased in isoproterenol-challenged rats as compared to that of rats in the control group. Treatment with ginsenoside Rg2 decreased the CK-MB activity and the troponin T level. This could be due to the protective effect of ginsenoside Rg2 on myocardium, preventing the cardiac damage and thereby restricting the leakage of CK-MB and troponin T from the myocardium into the blood stream.

The preliminary histopathological findings of the isoproterenol-challenged myocardium exhibited an infarcted zone with oedema, coagulative necrosis and inflammatory cells, and separation of cardiac muscle fibres. In the present study, the left ventricles from isoproterenol-treated rats showed widespread myocardial structure disorder and subendocardial necrosis with edema, and inflammatory cells. Treatment with ginsenoside Rg2 to the isoproterenol-challenged rats showed mild edema, less inflammatory cells and necrosis, which confirmed the cardioprotective property of ginsenoside Rg2.

Oxidative stress is believed to play a significant role in the initiation and progression of myocardial injury. The susceptibility of vascular cells to oxidative stress is a function of the overall balance between the degree of oxidative stress and the antioxidant defense capability. The increased generation of reactive oxygen species accompanied by depletion of the antioxidants in the defense system may contribute to oxidative stress and affect the pathogenesis of myocardial injury. As described earlier, increase in the MDA level was observed in the heart tissues after isoproterenol subcutaneous injection [21]. The increased level of MDA indicated an activation of the lipid peroxidative process, resulting in irreversible damage to the cardiac cells of animals subjected to isoproterenol. Free radical scavenging enzymes such as SOD and catalase were the cellular defense against oxidative stress, eliminating reactive oxygen radical, and preventing the formation of a more reactive radical. GSH was involved in a wide range of enzymatic reactions. A major function of GSH was to serve as a reductant in oxidation-reduction processes, which catalyze the transformation of peroxides and superoxide to

nontoxic species. GR, an indirect antioxidant enzyme, reduces oxidized glutathione to GSH [22]. In the present study, MDA content was significantly higher, while the GSH level and activities of SOD, catalase and GR were significantly lower in the isoproterenol group. Administration with ginsenoside Rg2 to the isoproterenol-challenged rats not only reduced MDA content, but also increased the GSH level and activities of SOD, catalase and GR. The findings indicated that the cardioprotective property of ginsenoside Rg2 was associated with its antioxidant activity.

Inflammation has been recognized as a critical factor in cardiovascular disease, and numerous anti-inflammatory drugs have been demonstrated to be effective in attenuating myocardial injury [5]. A previous study showed that IL-1β and TNF-α were highly expressed in the isoproterenol-induced myocardial injury rats [23]. It also reported that expressions of IL-1β and TNF-α were responsible for the activation of inflammatory cascade and subsequent aggravation of myocardial injury. Oxidative stress was a powerful stimulant for the expression of pro-inflammatory cytokines, which can result in further myocardial injury and oxidative stress [24]. This study showed that IL-1β and TNF-α were increased in the myocardium of isoproterenol-challenged rats. The observed increase of the pro-inflammatory cytokine might be due to oxidative stress induced by isoproterenol administration. Treatment with ginsenoside Rg2 decreased the level of the pro-inflammatory cytokines in the isoproterenol-induced myocardial injury rats. The findings indicated that ginsenoside Rg2 prevents myocardial injury by reducing oxidative stress, thereby decreasing the levels of pro-inflammatory cytokines and inhibiting inflammation.

In summary, we demonstrated for the first time that ginsenoside Rg2 attenuated myocardial injury by inhibiting inflammatory cytokines and reducing oxygen stresses on isoproterenol-challenged rats. However, our research is still insufficient, and further research about its action is warranted.

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Disclosure of conflict of interest

None.

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